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A Ni^{II}-Containing Vitamin B_{12} Derivative with a Cofactor-F430-Type π-System

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Abstract: F430 is a unique enzymatic cofactor in the production and oxidation of methane by strictly anaerobic bacteria. The key enzyme methyl coenzyme M reductase (MCR) contains a hydroporphinoid nickel complex with a characteristic absorption maximum at around 430 nm in its active site. Herein, the three-step semi-synthesis of a hybrid Ni^{II}-containing corrinoid from vitamin B_{12} is presented, that partly resembles structural and spectroscopic features of F430. Key step of the route is the simultaneous demetallation and ring closure reaction of a 5,6-secocobalamin to metal-free 5,6-dihydroxy-5,6-dihydro-hydrogenobalamin with cobaltocene and KCN under reductive conditions. Studies on the coordination chemistry of the novel compound support the earlier hypothesis why nature carefully selected a corphin over a corrin ligand in F430 for challenging Ni-catalysed biochemical reactions.

Introduction: Cobalamins (Cbls, Figure 1, left) and coenzyme F430 ("F430", Figure 1, right) represent the structural most complex cofactors of the uroporphinoid family.[1-4] The combination of the corrin ligand of vitamin B_{12} ("B_{12}", **1**, Figure 1, left) and the tetrahydrocorphin ligand of F430 with the redox active transition metal ions cobalt and nickel, respectively, has been selected by nature for catalyzing unique organometallic transformations in biological systems.[5, 6] Apart from the central metal ion, both cofactors differ in size as well as in the constitution of the porphinoid macrocycle.

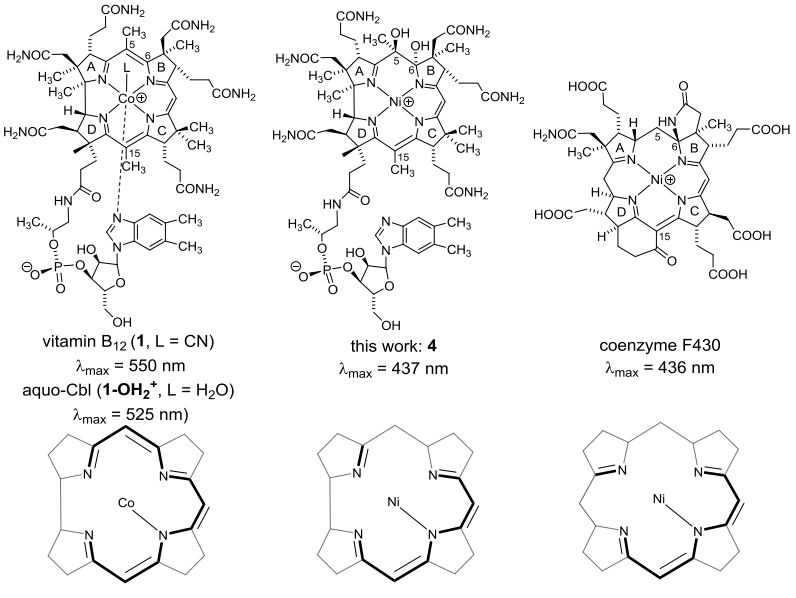


Figure 1. Comparison of the general structural formulas (top) and core structures (bottom) of (left) vitamin B_{12} and aquocobalamin (**1** and **1-OH_2**, counterion omitted), (right) coenzyme F430 and (middle) the new Ni^{II}-porphinoid **4**.

These structural differences have been addressed in seminal work of the groups of Eschenmoser and Thauer for obtaining insights into the question why nature chose these particular combinations for challenging C–H activation reactions.[2, 5, 7] Structural evidence of F430 was first obtained by comparing its characteristic absorptions with λ_{max} at ≈ 430 nm to those of synthetic Ni^{II}-containing hydrocorrin and hydrocorphin derivatives.[8] In contrast to the total synthesis of these macrocycles,[8-10] an alternative

semisynthetic approach to nickel-containing corrinoid derivatives starting from B_{12} is still lacking. New efforts in this pioneering area are of fundamental interest for a better understanding of similarities and differences in properties and reactivity of Ni- and Co-containing cofactors.[11-14] Applications in medicinal chemistry and environmental sensing can also be envisaged.[15-18]

In this study, we set out to synthesize and investigate modified B_{12} derivatives with a central Ni^{II}-ion and a corrin chromophore with 12 instead of 14 π electrons (Figure 1, middle), reminiscent to the core structure of cofactor F430 (Figure 1, right). The natural cofactor exhibits no conjugation between the pyrrolic subunits A and B and features an electron withdrawing ketone moiety at C15. In order to synthesize an analogous corrin ligand **3** with an interrupted π-system between C5 and C6 and additional electron-withdrawing substituents in the periphery of the macrocycle, we envisaged to oxidatively open the corrin macrocycle of B_{12} (**1**), extract the central Co-ion and close the resulting metal-free secocorrin to a diol derivative by reductive coupling (Scheme 1).[19-21] This "surgical intervention" seemed to be required, since all previous attempts to demetalate B_{12} without irreversible destruction of the corrin ring failed.[22, 23] Furthermore, we considered the one-electron reductant cobaltocene ($CoCp_2$) as a useful tool for efficient reduction of the Co^{III}- to a more labile Co^{II}-center as well as for reductive coupling of the two carbonyl units in a straightforward one-pot procedure.

Results and Discussion: The three-step synthetic route to the Ni^{II}-porphinoid **4** entails (a) an oxidative C–C double bond scission between C5 and C6 of the corrin macrocycle of B_{12} (**1**), (b) a one-pot reductive demetallation/ C–C bond forming reaction of $\mathbf{2}^-$ to the tetradentate metal-free macrocycle **3** and (c) its subsequent complexation with Ni^{II} ions (Scheme 1). Co_β,Co_α-dicyano-5,6-dioxo-5,6-secocobalamin $\mathbf{2}^-$ (isolated as **2-H**, Scheme S1) was synthesized according to literature procedures.[19, 21,24] Treatment of $\mathbf{2}^-$ with the one-electron reductant $CoCp_2$ (11 equiv.) in H_2O at pH 8.1 ([TRIS] = 0.1 M) resulted in a color change from orange to dark brown, indicating reduction of the Co^{III}-center to its Co^{II} state. Addition of excess KCN (107 equiv.) led then to the formation of a bright yellow compound (**3**; λ_{max} = 383 nm) in 63 % yield (isolated).[24] The luminescence properties of **3** (Figure 2) with intense fluorescence emission at 488 and 543 nm (λ_{ex} = 380 nm) suggested removal of the quenching cobalt ion from non-fluorescent $\mathbf{2}^-$.[25, 26]

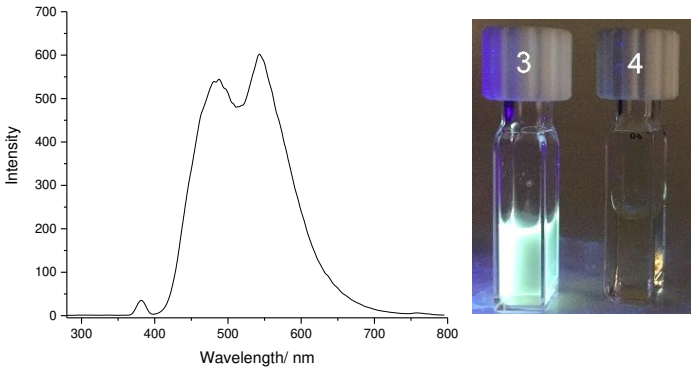
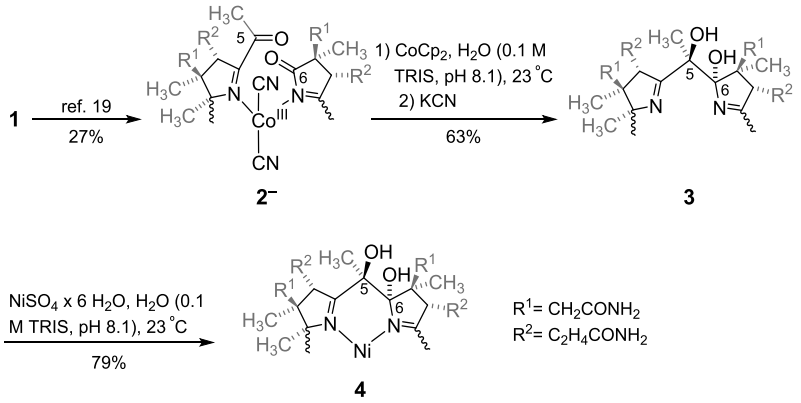


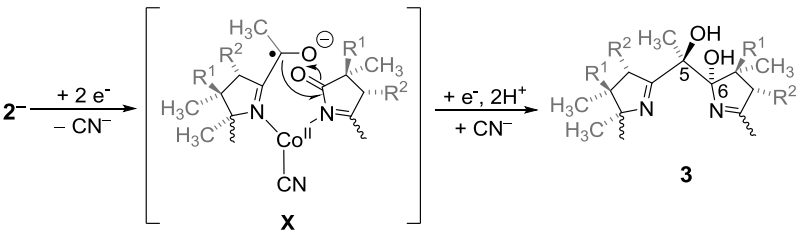
Figure 2. Left: Emission spectrum of **3** (0.05 mM in H_2O, λ_{ex} = 380 nm). Right: Difference in fluorescence emission between Ni^{II}-containing **4** and metal-free **3** when viewed under UV light (λ = 365 nm).

In the high-resolution mass spectrum of **3**, the M^- ion was observed at m/z = 1304.64348 indicating the molecular formula $C_{62}H_{91}N_{13}O_{16}P$ (m/z_{calc}: 1304.64498). These data are consistent with the formation of the metal-free porphinoid 5,6-dihydroxy-5,6-dihydro-hydrogenobalamin (**3**, Schemes 1 and 2). The tentatively assigned constitution of the reconstituted 5,6-dihydroxy-5,6-dihydrocorrin **3**[24] with a newly formed diol moiety at C5 and C6 was unequivocally confirmed later by thorough two-dimensional NMR studies of the corresponding Ni^{II}-complex **4**.



Scheme 1. Three-step synthetic route towards novel Ni^{II}-corrinoid **4** starting from B_{12} (**1**). Only the "northern" part of the corrin macrocycle is shown, charges omitted for clarity.

The mode of conversion of $\mathbf{2}^-$ with $CoCp_2$ and KCN to metal-free **3** is rationalized by reduction of the metal as well as two ligand-centered one-electron reductions within the molecule (Scheme 2).[27] It is suggested, that the kinetically inert Co^{III} d^6-low spin system is transformed into a more labile Co^{II} species in a d^7- configuration and the ketone moiety at C5 is converted to a reactive ketyl radical anion in intermediate **X** . The strongly δ-donating CN^- ions lead then to sequestration of the Co^{II}-ion from the macrocycle while the carbon centered radical attacks the lactam moiety, which undergoes a second one-electron reduction and subsequent protonation to form metal-free **3** (Scheme 2). Evidence from several control experiments support this mechanistic hypothesis. Without prior reduction with $CoCp_2$, demetallation of $\mathbf{2}^-$ with excess cyanide was not observed. The existence of the hypothetical intermediate **X** (Scheme 2) was supported by radical scavenging experiments. Only starting material $\mathbf{2}^-$ was observed by UPLC-MS analysis when the reductive coupling with $CoCp_2$ was attempted in the presence of the radical scavenger TEMPO.[24]



Scheme 2. Schematic representation of the proposed mechanism for the reductive demetallation/ C-C bond forming reaction from $\mathbf{2}^-$ to **3**, charges omitted for clarity.

Complexation of yellow-colored **3** (0.95 mM) with Ni^{II} (32 equiv.) to orange-colored 5,6-dihydroxy-5,6-dihydronibalamin (**4**; m/z = 1360.56121, m/z_{calc}: 1360.56468 for $C_{62}H_{89}N_{13}NiO_{16}P$ ($[M-H]^-$); isolated yield: 79%) in H_2O at pH 8.1 ([TRIS] = 0.1 M) led to quenching of the intrinsic fluorescence of **3** by the heavy metal effect of the Ni^{II} ion (Figure 2 right).[26, 28] The Vis region of the absorption spectrum (H_2O) of Ni^{II}-containing **4** indicated a maximum at 437 nm, reminiscent to that of cofactor F430 ($\Delta\lambda_{max}$ = 1 nm; Figures 1 and 3). Differences were observed in the UV region of the spectra. F430 exhibits an absorption at 275 nm with lower intensity compared to its maximum at 436 nm ($\varepsilon_{UV}/\varepsilon_{VIS}$ = 0.9). In contrast, derivative **4** exhibits an absorption maximum at 280 nm with an additional shoulder at 289 nm that is 1.7 times more intense than its αβ-band ($\varepsilon_{UV}/\varepsilon_{VIS}$ = 1.7). Despite these differences, **4** shows more similarities with the spectral features of F430 than with those of aquo-Cbl (**1-OH_2^+**; Figures 1 and 3). This observation is in agreement with earlier studies on synthetic nickel-dihydrocorrin and corphin complexes but has not been demonstrated so far for a semi-synthetic Ni^{II}-corrinoid derived directly from B_{12}.

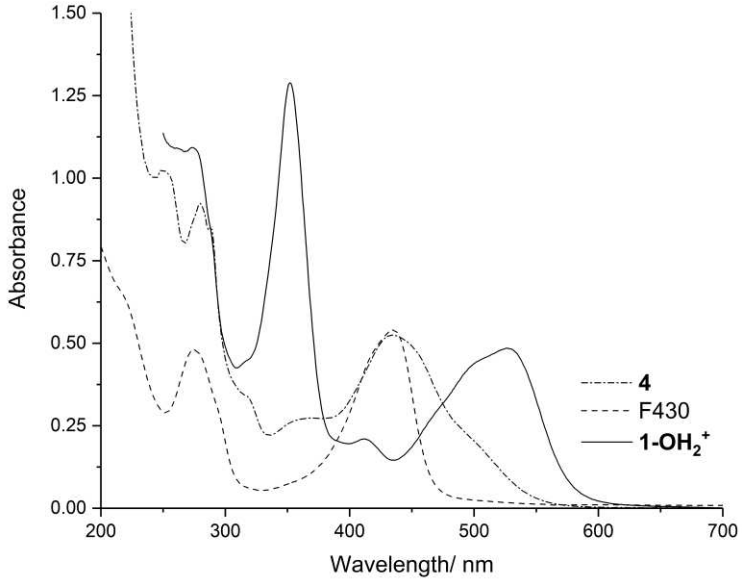


Figure 3. UV-Vis spectra of Ni^{II}-porphinoid **4** (dashed-dotted line, in H_2O), F430 (dashed line, mixture between penta- and hexa-coordinate high-spin forms in H_2O/MeCN 7:3)[29] and **1-OH$_2^+$** (solid line, in H_2O).

More detailed studies of the kinetics and thermodynamics of the complexation reaction were performed with UV-Vis spectroscopy. When $NiSO_4$ (10 equiv.) was added to a soln. of **3** in H_2O at pH 8.1 ([TRIS] = 0.1 M), clean conversion to **4** within 300 min was monitored spectroscopically by changes in the αβ-band (Figure 4). Fitting of the increasing absorption of **4** at 433 nm vs. time with a mono-exponential model (R^2 = 0.97) yielded a pseudo-first order rate constant (k_{obs}) of 2.15 ± 0.04 × 10^{-4} s^{-1}. A conditional binding constant (K_a) of 0.31 ± 0.12 × 10^3 M^{-1} at pH 8.0 and 300 K was obtained for this binding event from titrations of **3** with increasing concentrations of Ni^{II} (Figure S10)[24]. However, there is – to the best of our knowledge – no thermodynamic and kinetic data of metal ion incorporation into synthetic corrin or corphin ligands available.

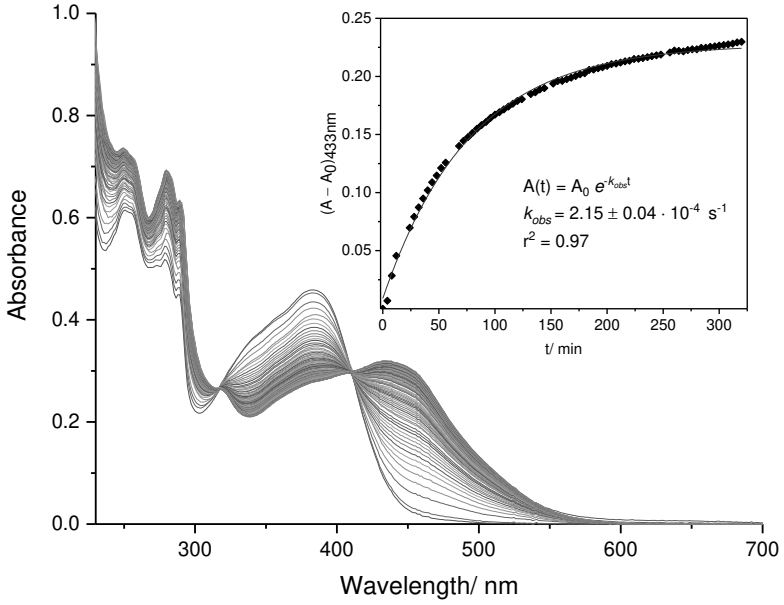


Figure 4. Spectral changes during formation of **4** from **3** in H_2O ([TRIS] = 0.1 M, pH 8.1) with $NiSO_4$ (10 equiv.). Insert: Plot of the change in absorbance at 433 nm vs. time.

^{1}H-NMR analysis of **4** in D_2O exhibited sharp, resolved signals indicating a diamagnetic, square-planar Ni^{II} complex in a d^8-configuration. The signals for the proton at C10 as well as the methyl group C1A were found at 6.02 and 1.69 ppm, respectively, i. e. with a significant low-field shift compared to H_2OCbl (**1-OH$_2^+$**, Figure S1 and Table S2).[24, 30] This behavior strongly supports a square-planar coordination geometry of **4** with an appended, non-coordinating dimethylbenzimidazole (Dmbz) base (Figures 1 and 5). A ^{1}H-^{13}C-HMBC spectrum showed correlations between the protons of the methyl group at C51 (2.69 ppm) and C6 (176.5 ppm). This correlation confirmed the reconstitution of the C5-C6 bond between rings A and B in **4**, introduced during reductive demetallation of the secocorrin **2** to **3** (Scheme 1 and Figure 5 Carbons associated with the "northern" part of the corrin macrocycle were detected at 201.4 (C4), 33.9 (C51) and 176.5 ppm (C6), respectively. The remarkable downfield shifts ($\Delta\delta$ = 9.7 – 17 ppm) compared to **1-OH$_2^+$** agree with the expected de-shielding effects of the two adjacent hydroxy groups at C5 and C6. Interestingly, the carbon shifts of 7 out of 14 remaining corrin core carbons of **4** (C2, C9, C10, C13, C15, C16 and C18), as well as of the *c*, *b* and *d*-side chains, are similar to those of F430 ($\Delta\delta \leq 5.7$ ppm, Table S1[24]).[31] The stereochemistry at positions C5 and C6 of the reconstituted macrocycle was addressed with ^{1}H-^{1}H-ROESY spectroscopy (Figure 5). The methyl group at C5 displayed strong NOE correlations to H_3C1A and other groups oriented towards the lower (α-) side of the corrin ligand, allowing for unequivocal location of the opposing OH group at the β-side (*R*-configuration). Unfortunately, all our attempts failed to elucidate the stereochemistry of the OH group at C6 by NMR spectroscopic methods. Evidence for the absolute configuration at C6 was then obtained by circular dichroism (CD) spectroscopy. The CD spectrum of metal-free **3** between 250 and 430 nm (Figure S3[24]) is (almost) identical to that of a yellow *5R*, *6S*-dihydroxy-corrinoid, but fundamentally different to that of a related *5R*, *6R* isomer (Figure S4[24]).[32, 33] Based on this comparison, we tentatively assign the OH group at C6 to be directed towards the α-side (*S*-configuration), i. e. in *trans* position to the adjacent OH group located at C5 (Figures 5 and S4[24]).

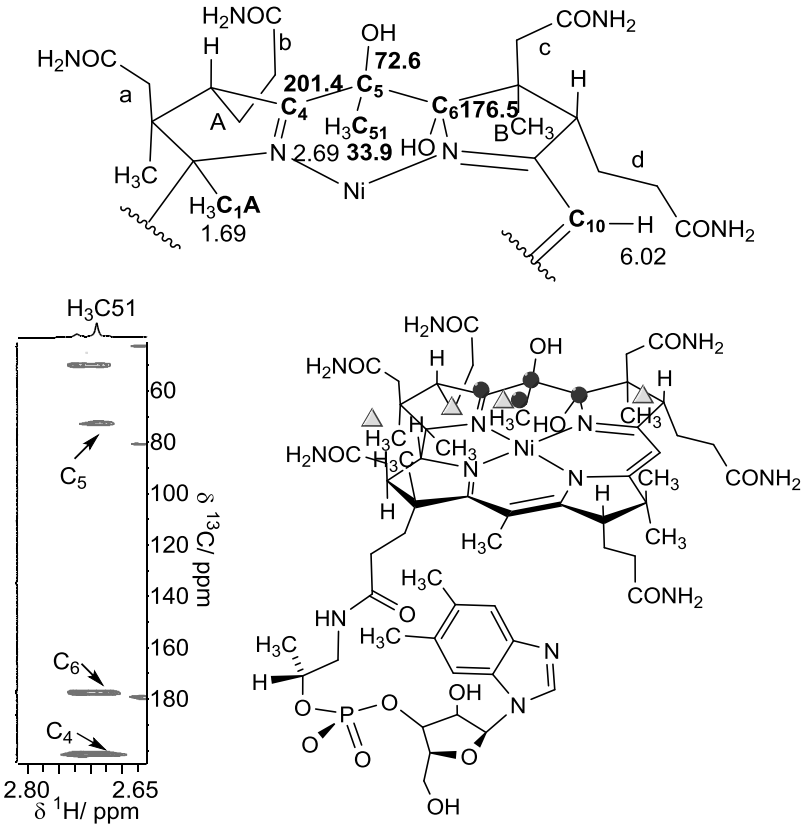


Figure 5. Top: significant ^{1}H-NMR shifts and ^{13}C-NMR shifts (bold) of carbons located at the "northern" part of the novel corrinoid ligand of **4**. Bottom, left: Section of the ^{1}H-^{13}C-HMBC spectrum of **4**, highlighting the correlations between the methyl group at C51 and core carbons C4 – C6. Bottom, right: Proposed solution structure of **4** according to 2D-NMR studies

(circles: key HMBC correlations, triangles: key NOE correlations between protons at C51 and the indicated carbons or protons, respectively).

In our studies, remarkable similarities in the UV-Vis and ^{13}C-NMR spectra of **4** and F430 were observed despite major structural differences in size and constitutional details of the two tetradentate macrocycles. The spectral resemblances are explained by some common structural features of the compounds (Figure 1). F430 and **4** both possess a 10 π-electron system delocalized between the B-C-D pyrrolic subunits of the macrocycles, an isolated double bond at ring A as well as electron-withdrawing groups at the periphery of the macrocycle.

In contrast to the spectral properties, differences in the axial coordination geometry of the compounds were observed. Square-planar **4** does not contain any additional axial ligands in coordinating solvents such as H_2O or DMSO as derived from NMR and spectrophotometric experiments. Spectral changes were also not observed in UV-Vis titrations upon addition of up to 100 equiv. of strongly nucleophilic CN^- or SCN^- (Figure S9[24]). In contrast, F430 forms paramagnetic penta- and hexa-coordinated species in H_2O, other co-ordinating solvents and upon addition of nucleophilic ligands.[9, 34]

Our observations on the properties and coordination chemistry of **4** are in line with earlier studies of the group of Eschenmoser on synthetic Ni^{II}-corrins and corphins.[8, 9] In these model compounds, a spin crossover from a square-planar geometry with a diamagnetic Ni^{II} ion to a paramagnetic octahedral geometry was only observed for Ni^{II} ions embedded in a corphin ligand rather than a corrin macrocycle. This important difference between corphins and corrins was attributed to a better fit of the (larger) paramagnetic Ni^{II}-ion into the larger coordination hole of the corphin ligand. Our studies with the novel semi-artificial Ni^{II}-containing B_{12} derivative **4** strongly support this hypothesis.[7]

In summary, a cobalamin derivative containing a Ni^{II} instead of a Co^{III} center was synthesized in three steps directly from vitamin B_{12}. Structural and spectroscopic properties of this semi-artificial "nibalamin" are partly similar to those of cofactor F430, while studies on the coordination chemistry support earlier findings with synthetic Ni^{II}-corrinoids, that a spin-crossover of the d^8-system from a square-planar to an octahedral geometry is not observed for Ni^{II}-corrins, in contrast to the related corphin-complexes. These findings are not only insightful regarding why particular combinations of metal ions (Ni vs. Co) with structurally complex porphinoid ligands (corphin vs. corrin) were chosen by nature for unique enzymatic reactions, but might as well bear the potential for future applications of semi-synthetic metallo-corrins in analytical and medicinal science.

Conflicts of interest: There are no conflicts to declare.

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Vitamin B12 F430 cofactor corrinoid biomimetic demetallation nickel cobaltocene semi-synthesis radical